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U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

Q/98394 US

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (known, see 37 CFR 1.5)

097744282

INTERNATIONAL APPLICATION NO.

PCT/EP99/05331

INTERNATIONAL FILING DATE

19-JUL-1999

PRIORITY DATE CLAIMED

23-JUL-1998

TITLE OF INVENTION

USE OF HC gp-39 IN IMMUNE DISEASES

APPLICANT(S) FOR DO/EO/US

MILTENBURG, Andreas M.M. and BOOTS, Anna M.H.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
 2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
 3. ☐ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
 4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
 5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
 6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☒ have not been made and will not be made.
 8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
 9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
 10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).
- Items 11 to 16 below concern document(s) or information included:**
11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
 12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
 13. ☒ A **FIRST** preliminary amendment.

☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
 14. ☐ A substitute specification.
 15. ☐ A change of power of attorney and/or address letter.
 16. ☐ Other items or information:

EXPRESS MAIL EL400659173US

U.S. APPLICATION NO. 09/144282 INTERNATIONAL APPLICATION NO. PCT/EP99/05331		ATTORNEY'S DOCKET NUMBER 0/98394 IIS					
17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) : Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =		<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <th style="width:60%;">CALCULATIONS</th> <th style="width:40%;">PTO USE ONLY</th> </tr> <tr> <td colspan="2" style="height: 100px;"></td> </tr> </table>		CALCULATIONS	PTO USE ONLY		
CALCULATIONS	PTO USE ONLY						
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td style="width:60%;">\$</td> <td style="width:40%;">860.00</td> </tr> <tr> <td>\$</td> <td></td> </tr> </table>		\$	860.00	\$	
\$	860.00						
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CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE				
Total claims	12 - 20 =		X \$18.00 \$				
Independent claims	2 - 3 =		X \$80.00 \$				
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00 \$				
TOTAL OF ABOVE CALCULATIONS =		\$ 860.00					
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.							
SUBTOTAL =		\$					
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).							
TOTAL NATIONAL FEE =		\$ 860.00					
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property			+				
TOTAL FEES ENCLOSED =		\$ 900.00					
		Amount to be refunded:	\$				
		charged:	\$ 900.00				

a. ☐ A check in the amount of \$_____ to cover the above fees is enclosed.

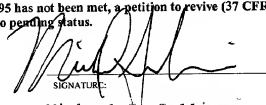
b. ☒ Please charge my Deposit Account No. 02-2334 in the amount of \$900.00 to cover the above fees.
 A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any
 overpayment to Deposit Account No. 02-2334. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR
 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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 SIGNATURE:
 Michael G. Sullivan
 NAME
 30,377
 REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

MILTENBURG, Andreas M.M. and BOOTS, Anna M.H.

Serial Number: To be assigned Group Art Unit: To be assigned

Filed: Concurrently herewith Examiner: To be assigned

For: USE OF HC gp-39 IN IMMUNE DISEASES

Corresponding to: PCT/EP99/05331, filed July 19, 1999

PRELIMINARY AMENDMENT

Assistant Commissioner of Patents
Washington, D.C. 20231

January 22, 2001

Sir:

Prior to the calculation of the fee in the above-identified application, please make the following amendments:

IN THE SPECIFICATION:

Page 1, above line 3, please insert the heading -- Field of the Invention --;

between lines 6 and 7, please insert the heading -- Background of the Invention --;

line 8, after "is" please insert -- , --;
line 10, delete "individuals" and insert -- individual's --;

line 11, after "is" please insert -- , --;
line 16, after "complex" please insert -- , --; and
line 30, after "disease" please insert -- such --.

Page 3, between lines 31 and 32, please insert the heading -- Summary of the Invention --.

Page 4, between lines 3 and 4, please insert the heading -- Detailed Description of the Invention --;

after line 3 and before the above heading, insert --

Brief Description of the Figures

Figures 1A - F show the effect of nasal application of 30 µg HC gp-39 on onset and progression of collagen type II induced arthritis in DBA/1 mice.

Figure 1A shows the effects on a clinical arthritis score in groups of mice

Figure 1B shows the effects on a clinical arthritis score in individual mice

Figure 1C shows the inhibition of histological alterations as evidenced by infiltrate score

Figure 1D shows the inhibition of histological alterations as evidenced by photographs of knee joints

Figure 1E shows the inhibition of radiological damage as evidenced by X-ray imaging

Figure 1F shows the inhibition of radiological damage as evidenced by photographs of ankle joints

Figure 2 shows the effect of nasal application of 3, 10 or 30 µg HC gp-39 on onset and progression of collagen type II induced arthritis in DBA/1 mice. The effects on a clinical arthritis in groups of mice are shown. --;

line 20, delete "effcet" and insert -- effect --; and

line 32, after "invention" please insert -- , --.

Page 7, line 14, please delete "of" and insert -- by --.

Page 8, line 21, delete "Pharmaceutical" and insert -- Pharmaceutically --;

line 24, after "example" insert -- , --;

line 25, after "addition" insert -- , --; and

line 30, after "Furthermore" insert -- , --.

Page 9, delete lines 15 - 29.

Page 10, line 1, delete "Examples".

Page 12, line 25, delete "succesfully" and insert
-- successfully --.

IN THE CLAIMS:

Please cancel claims 1 - 3 without prejudice or disclaimer of
the subject matter thereof.

Please add the following new claims 4 - 15.

-- 4. A method of treating an inflammatory autoimmune disease by
modulating the reactivity of lymphocytes associated with said
disease, comprising the step of administering a pharmaceutical
composition comprising an effective amount of HP gp-39 or
fragments thereof, and a pharmaceutically acceptable carrier,
wherein said lymphocytes are reactive to antigens other than HC
gp-39 which are present in the same tissue as HC gp-39. --

-- 5. The method of claim 4, wherein said fragments are
selected from one or more of SEQ ID NO:1(FGRSFTLAS), SEQ ID
NO:2(FTLASSETG), SEQ ID NO:3(YDDQESVKS), SEQ ID NO:4(FSKIASNTQ),
SEQ ID NO:5(PTFGRSFTLASSE), SEQ ID NO:6(RSFTLASSETGVG), SEQ ID
NO:7(VGYDDQESVSKSV) and SEQ ID NO:8(SQRFASKIASNTQSR). --

-- 6. The method of claim 5, wherein said fragments are selected
from one or more of SEQ ID NO:5(PTFGRSFTLASSE), SEQ ID
NO:6(RSFTLASSETGVG), SEQ ID NO:7(VGYDDQESVSKSV) and SEQ ID
NO:8(SQRFASKIASNTQSR). --

-- 7. The method of claim 4, wherein said autoimmune disease is
rheumatoid arthritis. --

-- 8. The method of claim 5, wherein said autoimmune disease is rheumatoid arthritis. --

-- 9. The method of claim 6, wherein said autoimmune disease is rheumatoid arthritis. --

-- 10. A method for modulating the reactivity of lymphocytes that are reactive to antigens other than HC gp-39 which are present in the same tissue as HC gp-39, comprising the step of administering a pharmaceutical composition comprising an effective amount of HC gp-39 or fragments thereof, and a pharmaceutically acceptable carrier, whereby the reactivity of said lymphocytes is modulated. --

-- 11. The method of claim 10, wherein said fragments are selected from one or more of SEQ ID NO:1(FGRSFTLAS), SEQ ID NO:2(FTLASSETG), SEQ ID NO:3(YDDQESVKS), SEQ ID NO:4(FSKIASNTQ), SEQ ID NO:5(PTFGRSFTLASSE), SEQ ID NO:6(RSFTLASSETGVG), SEQ ID NO:7(VGYDDQESVKS) and SEQ ID NO:8(SQRFSKIASNTQSR). --

-- 12. The method of claim 11, wherein said fragments are selected from one or more of SEQ ID NO:5(PTFGRSFTLASSE), SEQ ID NO:6(RSFTLASSETGVG), SEQ ID NO:7(VGYDDQESVKS) and SEQ ID NO:8(SQRFSKIASNTQSR). --

-- 13. The method of claim 10, wherein said autoimmune disease is rheumatoid arthritis. --

-- 14. The method of claim 11, wherein said autoimmune disease is rheumatoid arthritis. --

-- 15. The method of claim 12, wherein said autoimmune disease is rheumatoid arthritis. --

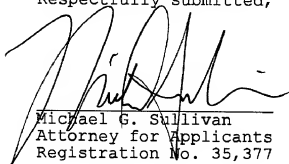
REMARKS

Claims 1 - 3 are canceled and claims 4 - 15 are added.
Claims 4 - 15 are presented for examination.

New claims 4 - 15 have been added to adapt the claim language to conventional U.S. patent practice and not for purposes of patentability. New claims 4 - 15 are not believed to narrow the scope of Applicants' invention.

It is believed that claims 4 - 15 recite a patentable improvement in the art. Favorable action is solicited. In the event any fees are required with this paper, please charge our Deposit Account No. 02-2334.

Respectfully submitted,



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11MILTENBURG-PRELIMINARY

09/744282

Use of HC gp-39 in immune diseases

The invention pertains to a treatment of (auto)immune disease. More specifically the invention is directed to employing Human Cartilage gp-39 (HC gp-39) as a modulator protein for therapy irrespective of the initial events or causes that led to the initiation of the (auto)immune condition.

The primary functional role of the immune system is to protect the individual against invading pathogens bearing foreign, that is non-self, antigens. In order to fulfill this function in a safe and effective manner, a mechanism is required to discriminate between foreign antigens and autoantigens derived from the individuals own body. Failure of this process of self-non-self discrimination, that is loss of immunological tolerance to self antigens, may lead to immune reactivity to autoantigens resulting in autoimmune disease, involving tissue damage and loss of organ function.

Autoimmune diseases are considered to be a major problem in human health care. Some autoimmune diseases may be the result of an immunological process directed at one antigen or antigenic complex whereas in others the autoimmune reaction may involve many types of antigens that may be present in multiple organs. Several lines of evidence have indicated that the immune system is involved in the pathology of autoimmune diseases. First, the chances of individuals to develop an autoimmune disease are closely linked to their genetic backgrounds: genes encoding major histocompatibility complex (MHC) class II molecules that present (auto)antigens to responding T cells which recognize MHC-peptide complexes show a strong genetic linkage to disease susceptibility. Second, cells of the immune system such as monocyte/macrophages and T cells infiltrate target organs. Third, T cells of patients with autoimmune diseases proliferate in vitro in response to potentially involved autoantigens. Fourth, studies in animal models of autoimmunity have unequivocally demonstrated that cells of the immune system such as monocyte/macrophages and T cells are involved in induction and expression of disease activity.

The immunopathology that may occur in case of an autoimmune disease can be illustrated by a disease as rheumatoid arthritis (RA). RA presents itself as a chronic multisystem disease in which the common clinical manifestation is the persistent inflammatory synovitis accompanied by proliferation of synovial cells, pannus

formation, cartilage degradation and bone erosion, and ultimately joint deformity resulting in loss of function.

Existing therapies for the treatment of autoimmune disorders, such as RA, in which the immune system generates an unwanted and undesirable inflammatory response, are inadequate. Treatment has focused on relief of symptoms of autoimmune disease rather than on its cause. Most drugs used in the treatment of autoimmune diseases, e.g. steroids and non-steroidal anti-inflammatory compounds, are nonspecific and have significant toxic side effects. This is especially problematic since autoimmune diseases are chronic conditions which require the prolonged administration of drugs.

Antigen-specific, nontoxic immunosuppression therapy provides a very attractive alternative for the nonspecific immunosuppression. This antigen-specific therapy involves the treatment of patients with the target autoantigen or with synthetic T cell-reactive peptides derived from the autoantigen. These synthetic peptides correspond to T cell epitopes of the autoantigen and can be used to induce specific T cell tolerance both to themselves and to the autoantigen. Although it seems paradoxical to desensitize the immune system with the very same antigen responsible for activating the immune system, the controlled administration of the target (auto)antigen can be very effective in desensitization of the immune system. Desensitization or immunological tolerance of the immune system is based on the long-observed phenomenon that animals which have been fed or have inhaled an antigen or epitope are less capable of developing a systemic immune response towards said antigen or epitope when said antigen or epitope is introduced via a systemic route.

The human cartilage glycoprotein-39 (HC gp-39) was previously identified as a target autoantigen in RA (Verheijden GFM et al., 1997, Arthritis Rheum., 40, 1115). The autoantigenic nature was demonstrated by selective recognition of DR4 (DRB1*0401) binding peptides by peripheral blood T cells from RA patients.

Using HC gp-39 as the inducing protein, in Balb/c mice a chronic, relapsing model of autoimmune-arthritis was developed. Following sensitization with a single, systemic injection with a low dose (μ g range) of HC gp-39 in incomplete Freund's adjuvant (IFA), clinical arthritis developed first in the fore paws. At a later stage, a chronic, second phase of arthritis occurred in the hind paws which waxes and wanes with broad peaks of activity and remained present for at least 250 days. Histological evaluation of hind paws showed marked infiltrates predominantly around the ankle joint. Safranin O staining

revealed depletion of proteoglycans indicating degradation of cartilage. The model was shown to be useful in the investigation of tolerance induction with HC gp-39 as a novel, specific therapy for RA. In this model, nasal application of μg amounts of HC gp-39 several days prior to induction prevented the onset of HC gp-39 induced arthritis. The mechanism behind this is thought to be T-cell mediated since a DTH response to HC gp-39 was completely abrogated. Therapeutic use of HC gp-39 was tested by tolerance induction after the first phase of arthritis had occurred. The data indicate that nasal inhalation of HC gp-39 leads to prevention of a broad peak of arthritis activity in the hind paws. Thus, it has been established that HC gp-39 can, in a highly effective manner, be used to induce antigen-specific immunological tolerance (WO 96/13517).

The main problem in (auto)immune diseases (such as e.g. RA) is that the precise targets or antigens that the immune system is adversely reacting to are largely unknown, implicating that modulating a disease entity in an antigen-specific fashion may not be possible.

It would be an important advantage, however, if an antigen-driven, nontoxic form of immunomodulation therapy could be utilized without knowledge of the antigen(s) that are involved as a target in the (auto)immune response. Such an antigen-driven therapy would involve the generation of antigen-specific modulator cells with the use of an antigen that is expected to be released or produced during the autoimmune process as a result of inflammation or tissue damage. In case of an autoimmune disease, the locally produced autoantigen could then activate or reactivate modulator cells induced with such an antigen.

Evidence indicates that HC gp-39 is being expressed under numerous inflammatory (auto)immune conditions including RA, osteoarthritis (OA), alcohol-induced liver fibrosis, inflammatory bowel disease (IBD) and systemic lupus erythematosus (SLE). In general, HC gp-39 is expected to be expressed in those immune conditions in which monocyte to macrophage maturation occurs (Krause SW et al, 1996, J. Leukoc. Biol. , 60, 540) indicating that potentially in all inflammatory autoimmune diseases HC gp-39 can be found. However, expression of HC gp-39 may not be the direct cause of disease, but the result of localized inflammation.

It has now been found that induction of HC gp-39 reactive modulator cells is beneficial under those conditions in which unwanted immunological activity results in the expression of HC gp-39. Therefore, mucosal administration of HC gp-39 is of

benefit in (auto)immune conditions in general and can be used in the absence of knowledge of the (auto)antigens that are involved in initiation of the inflammatory condition.

According to the present invention it is surprisingly found that HC gp-39 can effectively modulate disease activity irrespective of the antigen(s) that are involved as a target in the (auto)immune response. Thus, it has been found that the mere presence of HC gp-39 at a site of inflammation or in lymph nodes draining such a site, for instance as the result of production during the process of monocyte to macrophage maturation, can result in the activation of modulatory T-cells induced with the use of HC gp-39, and therefore result in downmodulation of disease activity.

It has been demonstrated by Zhang JZ et al (1990, J. Immunol. 145, 2489) that pretreatment of Lewis rats with collagen type II (CII) on days -7, -5 and -2 before induction of disease inhibited arthritis severity in an adjuvant arthritis model (AA model). A pronounced suppression of joint swelling occurred in rats that had been fed with a low amount of CII. This concentration of CII showed very limited effectivity in a treatment protocol (feeding rats three times a week starting at day 17 after arthritis induction) as generally observed in antigen-based immunotherapy of autoimmune diseases.

In contrast to the effect seen with CII, HC gp-39 does not downmodulate collagen induced arthritis when tested in a scheme designed to prevent the induction of this disease (application on days -15, -10 and -5). Thus, in this situation, HC gp-39 is not effective in downmodulation of arthritis activity when using a pretreatment protocol. Surprisingly, however, HC gp-39 is highly effective in treatment of the autoimmune condition induced with CII when given on days 20, 25 and 30 following arthritis induction. Therefore, when HC gp-39 was given in a therapeutic application schedule that is highly relevant to the clinical situation in which patients present themselves to the doctor with ongoing autoimmune disease, arthritis activity was strongly inhibited. This inhibition of arthritis activity as a result of application of HC gp-39 was much stronger than the effect seen with the antigen used in the induction of disease, collagen type II.

According to the invention HC gp-39 can be used to modulate lymphocytes that are reactive to antigens other than HC gp-39 but are present in the same tissue as HC gp-39. By the induction of antigen-specific T-cell tolerance, autoimmune disorders can be

treated by bystander suppression. More in general, the cells to be modulated are hematopoietic cells. In general, in order to function as a tolerogen the protein must fulfill at least two conditions i.e. it must possess an immune modulating capacity and it must be expressed locally or in lymph nodes draining a local site.

5 Thus, the present invention provides a method to treat patients suffering from inflammatory autoimmune diseases irrespective of the antigen involved as a target in the immune response, by administration of a pharmaceutical preparation comprising HC gp-39. Such patients may suffer from diseases like Graves' diseases, primary glomerulonephritis, osteoarthritis, juvenile arthritis, Sjögren's syndrome, myasthenia
10 gravis, rheumatoid arthritis, Addison's disease, primary biliary sclerosis, uveitis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis or diabetes. The polypeptide according to the present invention therefore can be used in the preparation of a pharmaceutical to induce tolerance in patients suffering from these diseases. Most preferred patients are treated which suffer from rheumatoid arthritis.

15 HC gp-39 or fragments thereof can thus be used for the manufacture of a pharmaceutical preparation for modulation of the reactivity of lymphocytes. These lymphocytes may be reactive to antigens other than HC gp-39. They are, however, present in the same tissue as HC gp-39. As a result inflammatory diseases are prevented.

Treatment of autoimmune disorders with HC gp-39 makes use of the fact that
20 bystander suppression is induced to unrelated but co-localized antigens. The regulatory cells secrete in an antigen specific fashion pleiotropic proteins such as cytokines which can downmodulate the immune response.

Optionally a treatment can be combined with the the administration of other medicaments such as DMARDs (Disease Modifying Anti-Rheumatic Drugs e.g.
25 sulfasalazine, anti-malarials (chloroquine, hydroxychloroquine) injectable or oral gold, methotrexate, D-penicillamine, azathioprine, cyclosporine, mycophenolate), NSAIDs (non steroidal anti inflammatory drugs), corticosteroids or other drugs knowns to influence the course of the disease in patients suffering from inflammatory diseases.

30 It will be clear that also fragments of the tolerogen having the relevant antigenic parts will be sufficient in downregulation of the immune response. Such fragments can be identified by the same assay as described in examples 1 and 2.

The term "fragment" refers to any sequence of amino acids that is part of the polypeptide defined above, having common elements of origin, structure and mechanism of action that are within the scope of the present invention and which are functionally equivalent to the whole antigen.

- 5 As used herein, "functional equivalent" means a compound having variations of HC gp-39 or fragments thereof while still maintaining functional i.e. immunological or tolerogenizing characteristics of the sequence of HC gp-39 or the epitope fragments.

- The variations that can occur in a sequence may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, 10 inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions that are expected not to essentially alter biological and immunological activities, have been described. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val (see Dayhof, M.D., Atlas of protein sequence and structure, Nat. 15 Biomed. Res. Found., Washington D.C., 1978, vol. 5, suppl. 3). Based on this information Lipman and Pearson developed a method for rapid and sensitive protein comparison (Science 227, 1435-1441, 1985) and determining the functional similarity between homologous polypeptides.

- 20 The preparation of the peptides according to the invention is effected by means of one of the known organic chemical methods for peptide synthesis. HC gp-39 and the peptides can also be prepared with the aid of recombinant DNA techniques. For this purpose, a nucleic acid sequence which codes for HC gp-39 or a peptide according to the invention or a multimer of said peptide is inserted into an expression vector. Suitable 25 expression vectors are, amongst others, plasmids, cosmids, viruses and YAC's (Yeast Artificial Chromosomes) which comprise the necessary control regions for replication and expression. The expression vector can be brought to expression in a host cell. Suitable host cells are, for instance, bacteria, yeast cells and mammalian cells. Such techniques are well known in the art (Sambrook et al., Molecular Cloning: a Laboratory 30 Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).

The peptides may be stabilised by C- and/or N- terminal modifications, which will decrease exopeptidase catalysed hydrolysis. The modifications may include: C-terminal

acylation, (e.g. acetylation = Ac-peptide), N-terminal amide introduction, (e.g. peptide-NH₂) combinations of acylation and amide introduction (e.g. Ac-peptide-NH₂) and introduction of D-amino acids instead of L-amino acids (Powell et al., J. Pharm. Sci., 81:731-735, 1992).

- 5 Other modifications are focussed on the prevention of hydrolysis by endopeptidases. Examples of these modifications are: introduction of D-amino acids instead of L-amino acids, modified amino acids, cyclisation within the peptide, introduction of modified peptide bonds, e.g. reduced peptide bonds $\psi[\text{CH}_2\text{NH}]$ and e.g. peptoids (N-alkylated glycine derivatives) (Adang et al, Recl. Trav. Chim. Pays-Bas, 113:63-78, 1994 and Simon et al, Proc. Natl. Acad. Sci. USA, 89:9367-9371, 1992).

- 15 The controlled administration of HC gp-39 and/or peptides comprising a subsequence of HC gp-39 can be effective in modulation of the immune system. According to the invention, patients in which the tissue is under attack of autoreponsive T cells can be treated with a pharmaceutical composition comprising HC gp-39, or one or more peptides according to the invention and a pharmaceutical acceptable carrier in order to generate T cells in these patients that exert e.g. bystander suppression in such a way that the inflammatory response is diminished.

- 20 Very suitable peptides to be used in a pharmaceutical composition according to the invention are the peptides comprising an amino acid sequence given in SEQ ID NO:1 (FGRSFTLAS), SEQ ID NO:2 (FTLASSETG), SEQ ID NO:3 (YDDQESVKS), SEQ ID NO:4 (FSKIASNTQ), SEQ ID NO:5 (PTFGRSFTLASSE, SEQ ID NO:6 (RSFTLASSETGVG), SEQ ID NO:7 (VGYYDDQESVKS) and SEQ ID NO:8 (SQRFKIASNTQSR) of WO 96/13517.

- 25 Suitable peptides according to the invention are the peptides comprising the SEQ ID NOs 1-8 flanked by sequences up to a total length of 55 amino acids. More preferably the peptides have a length of 25 amino acids. Even more preferably the amino acid sequence of the peptides is identical to the sequence of SEQ ID NO 1-8.

- 30 Proteins related to HC gp-39 can similarly be used to develop an autoimmune response. Therefore, it is to be expected that these proteins can also be used for subsequent tolerization. Suitable proteins to be used as an alternative for HC gp-39 in a pharmaceutical composition according to the invention are for example pig heparine-

binding 38kDa protein, bovine 39 kDa whey protein, human YKL-39 protein, murine breast regressing 39kDa protein (brp39), human oviduct-specific glycoprotein, murine oviduct-specific glycoprotein, hamster oviduct-specific glycoprotein, bovine oviduct-specific glycoprotein, human chitotriosidase precursor protein and murine secretory protein YM-1 precursor.

Also very suitable to be used in a pharmaceutical composition according to the invention are DNA (expression)vectors comprising DNA which encodes for HC gp-39 or one or more of the peptides or proteins according to the invention. Upon delivery the DNA (expression)vector can provide by expression a level of the recombinant HC gp-39 protein or fragments thereof according to the invention which is similar to the level which would be achieved by direct administration of a pharmaceutical composition comprising the HC gp-39 protein or peptides.

Tolerance can be attained by administering high or low doses of the tolerogen or peptides according to the invention. The amount of tolerogen or peptide will depend on the route of administration, the time of administration, the age of the patient as well as general health conditions and diet.

In general, a dosage of 0.01 to 10000 µg of peptide or protein per kg body weight, preferably 0.05 to 2000 µg, more preferably 0.1 to 100 µg of peptide or protein can be used.

Pharmaceutical acceptable carriers are well known to those skilled in the art and include, for example, sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil and water. Other carriers may be, for example MHC class II molecules, if desired embedded in liposomes.

In addition the pharmaceutical composition according to the invention may comprise one or more adjuvants. Suitable adjuvants include, amongst others, aluminium hydroxide, aluminium phosphate, amphigen, tocopherols, monophosphoryl lipid A, muramyl dipeptide and saponins such as Quil A. The amount of adjuvant depends on the nature of the adjuvant itself.

Furthermore the pharmaceutical composition according to the invention may comprise one or more stabilizers such as, for example, carbohydrates including sorbitol, mannitol, starch, sucrosedextrin and glucose, proteins such as albumin or casein, and buffers like alkaline phosphates.

Suitable administration routes are intramuscular injections, subcutaneous injections, intravenous injections or intraperitoneal injections, oral and intranasal administration. Oral and intranasal administration are preferred administration routes. Especially, modulator cells specific for the antigen could be generated by applying the antigen via the mucosae, for instance the nasal mucosae. Mucosal administration of antigens has been shown to induce immunological tolerance to such antigens.

The present invention demonstrates that HC gp-39 can be used to downmodulate autoimmune disease induced with a non-related antigen, thereby supporting the concept that HC gp-39 induced modulator cells can influence autoimmune processes.

The following examples are illustrative for the invention and should in no way be interpreted as limiting the scope of the invention.

Legends to the figures

Figure 1: Effect of nasal application of 30 µg HC gp-39 on onset and progression of collagen type II induced arthritis in DBA/1 mice.

- a. Effects on a clinical arthritis score in groups of mice
- b. Effects on a clinical arthritis score in individual mice
- c. Inhibition of histological alterations as evidenced by infiltrate score
- d. Inhibition of histological alterations as evidenced by photograph of knee joint
- e. Inhibition of radiological damage as evidenced by X-ray imaging
- f. Inhibition of radiological damage as evidenced by photographs of ankle joints

Figure 2: Effect of nasal application of 3, 10 or 30 µg HC gp-39 on onset and progression of collagen type II induced arthritis in DBA/1 mice. Effects on a clinical arthritis score in groups of mice are shown.

Examples

Example 1: Modulation of collagen-induced arthritis by nasal administration of 30 µg of HC gp-39

5 METHODS

Reagents

Bovine collagen type II was isolated from articular cartilage of knee joints obtained from 1-2 year old calves (Miller EJ, Rhodes RK. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology, Vol 82. New York: Academic Press, 1982:33-65). Collagen
10 was resolved in 0.05M HAc (5 mg/ml) and stored at -70°C. HC gp-39 was isolated from the supernatant of transfected CHO cells. HC gp-39 was purified from the culture supernatant by heparin affinity chromatography followed by superdex 75 chromatography. Purity was checked by SDS-PAGE. The control protein ovalbumin was from Sigma, St. Louis, USA.

15

Induction and modulation of arthritis

Male DBA/1 mice were obtained from Bomholtgaard. Mice were immunized (day 0) with 100 µg bovine collagen type II in complete Freund's adjuvant (CFA). Mice received an intraperitoneal booster injection with 100 µg bovine collagen type II in
20 saline. On days 20, 25 and 30 mice were treated via the intranasal pathway with either HC gp-39 (n=11; 30 µg/animal/dose), bovine collagen type II (n=11; 100 µg/animal/dose), control protein (n=10; ovalbumin; 100 µg/animal/dose) or with buffer alone (n=10). Nasal tolerance induction was performed under Enflurane anesthesia using a PT45 micro conduit and a Hamilton syringe. A control group (n=10) that was not
25 treated via the nasal pathway was also included in the experiment.

Clinical course of arthritis

Progression of arthritis activity was followed visually over time (days 23, 25, 27, 29, 31 and 34 following arthritis induction) and a score for severity of the disease was
30 given (macroscopic score based on redness and/or swelling in digits and/or paws). At later time points ankylosis was also included in this scoring system. Clinical severity of arthritis was graded on a scale of 0 to 2 for each paw according to the presentation of

redness and/or swelling; score 0: no changes; score 0.5: significant changes; score 1.0: moderate changes; score 1.5: marked changes; score 2.0: severe arthritis accompanied by maximal swelling and redness and later on ankylosis. A further refinement of this score (0.25 increments in the scoring system) has been implemented.

5

Histopathology

Knee joints were evaluated for the presence of histopathological abnormalities. Joints were removed in toto and fixed for 4 days in 4% formalin. After decalcification in 5% formic acid the specimens were processed for paraffin embedding. Tissue sections (7 µm) were stained with haematoxylin and eosin (HE) in order to detect inflammatory changes or with Safranin O to detect proteoglycan depletion indicative of cartilage breakdown. Histopathological changes were scored according to the following parameters. Infiltration of cells, on a scale of 0 to 3, was assessed based on the amount of inflammatory cells in the synovial cavity and the synovial tissues. Proteoglycan depletion was determined using Safranin O staining, and loss of proteoglycans was scored on a scale of 0 to 3, ranging from fully stained cartilage to destained cartilage or complete loss of articular cartilage. A further score for the progressive loss of articular cartilage, a characteristic parameter of collagen type II induced arthritis, is based on cartilage destruction. This destruction was graded on a scale of 0 to 3, ranging from the appearance of dead chondrocytes (empty lacunae) to complete loss of articular cartilage. Histopathological changes in the knee joints were scored in the patella/femur region on five semi-serial sections of the joint. For the ankle joint, the calcaneus region was scored.

25

Radiology

Radiography (X-ray imaging) was performed on hind paws of individual mice at the end of the experiment (day 34). Radiographs were scored with the use of a stereomicroscope under low magnification. A score of 0 to 5 was given to each paw according to the following guidelines: score 0: no changes; score 1: minor changes; score 2: moderate changes; score 3: marked changes; score 4: severe changes; score 5: complete destruction reflecting the severe arthritis that was externally visible in a number of animals.

30

RESULTS

Immunomodulatory activity of HC gp-39: clinical effects

DBA/1 mice immunized with collagen type II developed collagen-induced arthritis as planned and expected. Mice treated with the antigen used to induce disease, that is collagen type II, showed a trend towards milder disease activity in the earlier phase of the treatment. Mice treated with HC gp-39 were strongly inhibited in their expression of clinical disease activity (Figure 1A). Inhibition of clinical severity of arthritis was first detectable at day 25 of the experiment and lasted until day 34, at the end of the experiment. Only four of the eleven animals treated with HC gp-39 developed an arthritis that was moderate or severe, seven of the eleven mice were more or less protected (Figure 1B). Mice treated with ovalbumin or buffer showed clinical arthritis that was comparable with the clinical arthritis seen in control mice (Figures 1A and 1B). Thus, the experiment demonstrates that treatment with HC gp-39 can trigger modulatory or regulatory mechanisms that interfere with the induction of arthritis with the use of a non-related antigen, that is collagen type II. Therefore it can be concluded that (auto)immune conditions in which the inciting antigen(s) is not known may benefit from therapeutic application of HC gp-39.

Histological and radiological evaluation

Histological examination of knee joints revealed that inflammatory activity was strongly reduced in HC gp-39 treated animals (Figures 1C and 1D). Proteoglycan depletion, as evidenced by a reduction in staining with Safranin O, was strongly inhibited in HC gp-39 treated animals, as was the breakdown of articular cartilage (Figures 1C and 1D). Examination of radiographs revealed inhibition of cartilage breakdown and bone erosion in those animals that were successfully treated with HC gp-39 (Figures 1E and 1F). It is concluded that the therapeutic application of HC gp-39 not only modifies the clinical pattern of disease expression but also prevents ongoing histologically and radiographically detectable alterations that reflect inflammatory and erosive processes that lead to joint destruction and loss of function. Thus, treatment with HC gp-39 actually alters the course of the disease.

Example 2: Modulation of collagen-induced arthritis by nasal administration of 3, 10 or 30 µg of HC gp-39

METHODS

Reagents

Bovine collagen type II was isolated from articular cartilage of knee joints obtained from 1-2 year old calves (Miller EJ, Rhodes RK. In: Colowick SP, Kaplan NO, eds. Methods in Enzymology, Vol 82. New York: Academic Press, 1982:33-65). Collagen was resolved in 0.05M HAc (5 mg/ml) and stored at -70°C. HC gp-39 was isolated from the supernatant of transfected CHO cells. HC gp-39 was purified from the culture supernatant by heparin affinity chromatography followed by superdex 75 chromatography. Purity was checked by SDS-PAGE. The control protein ovalbumin was from Sigma, St. Louis, USA.

Induction and modulation of arthritis

Male DBA/1 mice were obtained from Bomholtgaard. Mice were immunized (day 0) with 100 µg bovine collagen type II in complete Freund's adjuvant (CFA). Mice received an intraperitoneal booster injection with 100 µg bovine collagen type II in saline. On days 20, 25 and 30 mice were treated via the intranasal pathway with either 3 µg/animal/dose, 10 µg/animal/dose or 30 µg/animal/dose of HC gp-39 (n=10 per group), bovine collagen type II (n=10; 100 µg/animal/dose) or control protein (n=10; ovalbumin; 100 µg/animal/dose). Nasal tolerance induction was performed under Enflurane anesthesia using a PT45 micro conduit and a Hamilton syringe.

Clinical course of arthritis

Progression of arthritis activity was followed visually over time (days 23, 25, 26, 28 and 30 following arthritis induction) and a score for severity of the disease was given (macroscopic score based on redness and/or swelling in digits and/or paws). At later time points ankylosis was also included in this scoring system. Clinical severity of arthritis was graded on a scale of 0 to 2 for each paw according to the presentation of redness and/or swelling: score 0: no changes; score 0.5: significant changes; score 1.0: moderate changes; score 1.5: marked changes; score 2.0: severe arthritis accompanied by maximal swelling and redness and later on ankylosis. A further refinement of this score (0.25 increments in the scoring system) has been implemented.

RESULTS

Immunomodulatory activity of various doses of HC gp-39: clinical effects

DBA/1 mice immunized with collagen type II developed collagen-induced arthritis as planned and expected. Mice treated with the antigen used to induce disease, that is collagen type II, again showed a trend towards milder disease activity in the earlier phase of the treatment. Importantly, mice treated with 30 μg of HC gp-39 were strongly inhibited in their expression of clinical disease activity (Figure 2). Mice that had been treated with 3 or 10 μg of HC gp-39 also showed inhibition in their expression of clinical disease, although to a lesser extent. Inhibition of clinical severity of arthritis is further detailed by data of hind paws of individual mice experiencing arthritis on evaluation days 23, 25 and 30, again demonstrating clinical effectiveness of treatment with HC gp-39, most effectively at the 30 μg /animal dose. Therefore, the experiment demonstrates that treatment with HC gp-39, especially with 30 μg /animal/dose, can trigger modulatory or regulatory mechanisms that interfere with the induction of arthritis with the use of a non-related antigen, that is collagen type II. Thus, it can be concluded that (auto)immune conditions in which the inciting antigen(s) is not known may benefit from therapeutic application of HC gp-39.

Example 3: HC gp-39 responses in RA patients

METHODS

Patients and reagents

Patients were diagnosed as suffering from RA according to the American Rheumatism Association (ARA) criteria (Arnett et al., Arthritis Rheum 31:315, 1988). Severity of disease of these patients was ranged from stage 0 - IV as determined by X-ray score. HC gp-39 was purified from culture supernatant by affinity chromatography followed by superdex 75 chromatography. Purity was checked on SDS-PAGE. Candida albicans was obtained from Hal allergenen lab.

Proliferation assay

Peripheral blood mononuclear cells (PBMC) were collected from heparinized venous peripheral blood by standard centrifugation on a Ficoll-Paque gradient. The assay used to determine proliferative responses to HC gp-39 is a modification of an

assay that had been described in the literature (Salvat et al., J Immunol 153:5321, 1994). In brief, PBMC obtained as described above were suspended in wells of a 24 well plate in a concentration of 5×10^5 cells per ml. Cells were incubated in medium alone or in the presence of antigen (HC gp-39 was tested in a dose range of 2, 10, 25 and 50 $\mu\text{g/ml}$; Candida albicans, a control antigen, was tested at 1 and 10 $\mu\text{g/ml}$). Spontaneous proliferation or background proliferation was assayed in three different background wells. Cultures were incubated for 6 days at 37°C in a humidified atmosphere of 5% CO_2 . Cells were then suspended and 200 or 150 μl volumes of medium were distributed in 4 or 5 fold in wells of a 96-well round-bottomed plate. Cultures were pulsed with 0.5 μCi (1.85×10^4 Bq) [^3H]thymidine ([^3H]TdR) for the last 18 hr of culture. Cells were harvested on glassfibre filters and [^3H]TdR incorporation was measured by gasscintillation. Note that counting by gasscintillation is fivefold less efficient compared to liquid scintillation. Filters were measured for 5 min (Packard Matrix 96 β -counter; Meriden, CT).

RESULTS

Responses of RA patients to HC gp-39

It was tested whether PBMC obtained from patients with RA ($n=10$) proliferated to HC gp-39. In order to establish the quality of the PBMC preparations under study, in a number of cases Candida albicans was used as a positive control antigen. Six out of ten patients could be classified as being a HC gp-39 responder ($\text{SI} > 10$); four out of ten patients did not respond to HC gp-39 ($\text{SI} < 10$) (Table 1). Both responders (R) and non-responders (NR) were found in various stages of the disease, either having active or non-active disease at the time of blood sampling. Thus, RA patients can be classified into individuals responding to HC gp-39 and individuals not responding to HC gp-39 with the use of a routine proliferation assay.

Table 1: HC gp-39 reactivity in RA patients

Patient ID	disease stage	BG	HC gp-39 ($\mu\text{g/ml}$)				<i>Candida</i> ($\mu\text{g/ml}$)		
			(2)	(10)	(25)	(50)	(1)	(10)	R/NR
246-0.2	Iiact	166	2.7	36	61	64	Nd	nd	R
308-0.1	IIIact	200	1.3	58	27	12	Nd	nd	R
403-3	IIIact	193	1.2	95	55	59	Nd	nd	R
406-0.1	Iiact	339	2.4	1.4	2.3	nd	147	126	NR
407-0	II	120	20	112	139	100	Nd	nd	R
421-0	0act	440	2.6	3.5	46	69	Nd	nd	R
438-0.2	IV	212	0.8	1	0.8	0.7	20	160	NR
464-0	0act	144	0.9	167	228	184	89	21	R
470-0.1	0act	113	1.4	1.1	2.5	nd	368	350	NR
474-0	IIIact	341	4.5	4.2	2.2	nd	70	55	NR

Disease stage is defined by Steinbrocker criteria. act = active disease if 1 or more joints are inflamed. BG is background counts per 5 minutes. Responses to HC gp-39 are given as SI values. SI values > 5 are considered positive and are indicated in bold. R = responder. NR = non responder. As a control, the response to *Candida albicans* was determined in order to establish the quality of the PBMC used. nd = not determined

Claims

1. Use of HC gp-39 or fragments thereof for the manufacture of a pharmaceutical preparation for modulation of the reactivity of lymphocytes, said lymphocytes
5 being reactive to antigens other than HC gp-39, said antigens being present in the same tissue as HC gp-39 so as to prevent inflammatory diseases.
2. Use of fragments according to claim 1 characterized in that the fragments are selected from the group comprising an amino acid sequence given in SEQ ID
10 NO:1 (FGRSFTLAS), SEQ ID NO:2 (FTLASSETG), SEQ ID NO:3 (YDDQESVKS), SEQ ID NO:4 (FSKIASNTQ), SEQ ID NO:5 (PTFGRSFTLASSE, SEQ ID NO:6 (RSFTLASSETGVG), SEQ ID NO:7 (VGYYDDQESVKSKV) or SEQ ID NO:8 (SQRFSKIASNTQSR).
3. Use of HC gp-39 or fragments thereof according to claims 1 or 2 for the
15 manufacture of a pharmaceutical preparation for modulation of the reactivity of lymphocytes, said lymphocytes being reactive to antigens other than HC gp-39, said antigens being present in the same tissue as HC gp-39 so as to prevent rheumatoid arthritis.

09/744282

SEQUENCE LISTING

<110> MILTENBURG, Andreas M.M.
BOOTS, Anna M.H.

<120> USE OF HC gp-39 IN IMMUNE DISEASES

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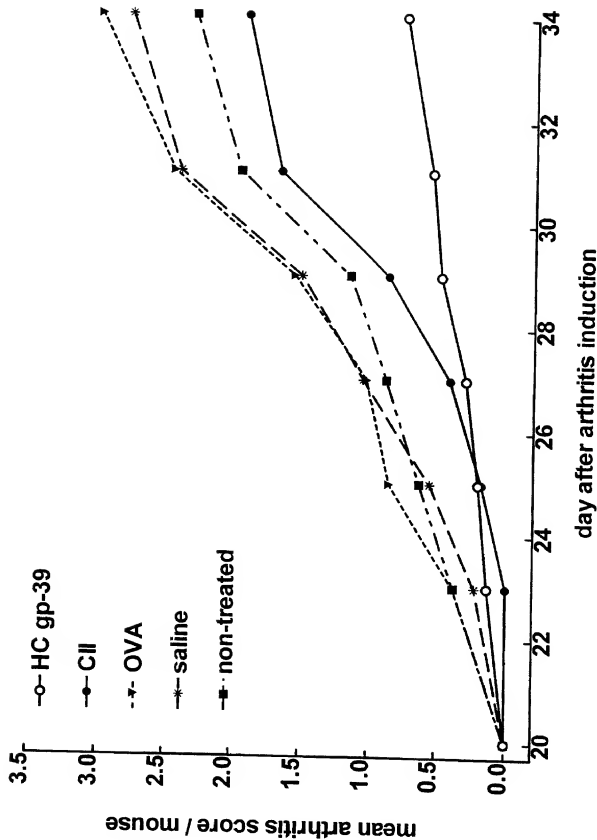
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Figure 1A

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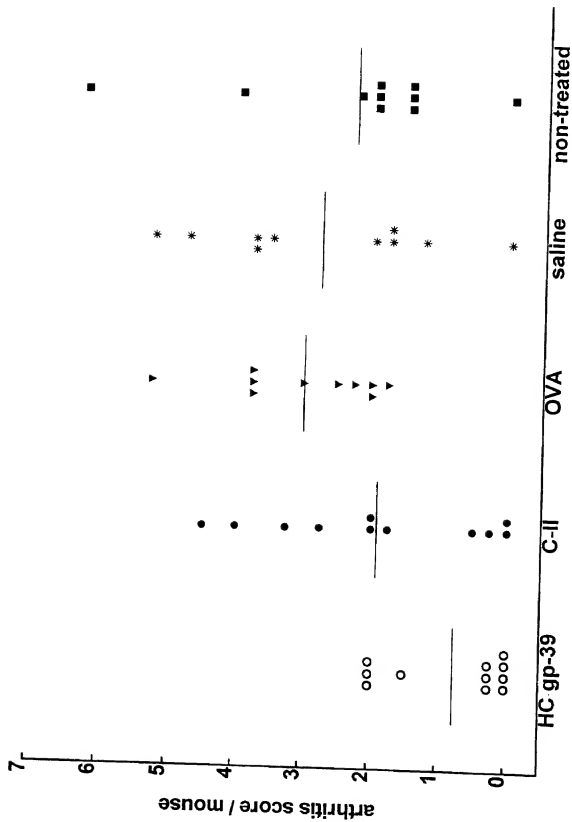
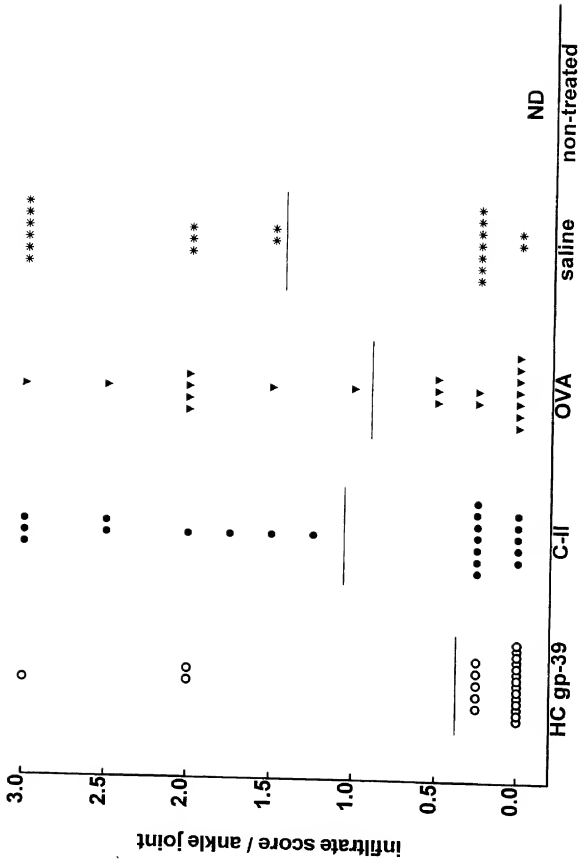


Figure 1B

SCANNED, # 60

Figure 1C



SCANNED, # 6

Figure 1D

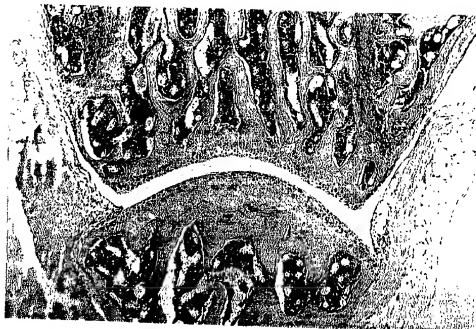


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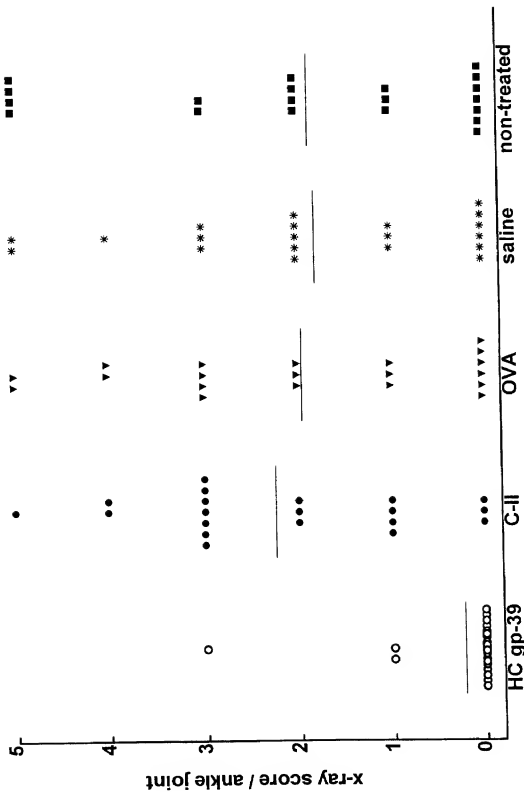
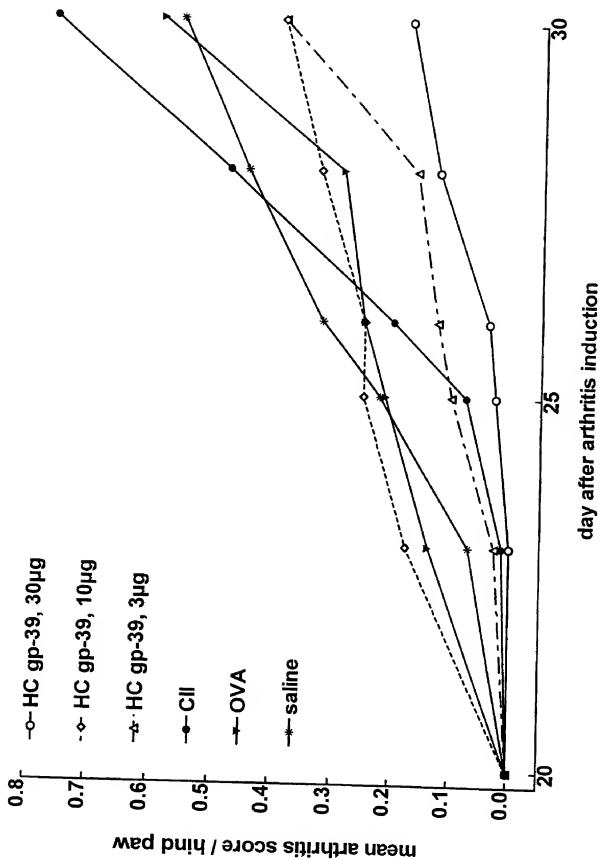
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Figure 1F



Figure 2



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09/744282

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter for which a patent is sought on the invention entitled:

"USE OF HC gp-39 IN IMMUNE DISEASES"

the specification of which

[CHECK ONE]

☐ is attached hereto

☐ was filed on _____ as Application Serial No.

and was amended on _____

[if applicable]

☒ as filed under the Patent Cooperation Treaty on 19 July 1999

Serial PCT/EP99/05331 The United States of America being designated.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined Title 37, Code of Federal Regulations Section 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications(s) for patent or inventor's certificate having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)

98202471.3

EP

23 / 07 / 1998

Priority claimed

☒ Yes ☐ No

Number

Country

Day/Month/Year filed

☐ Yes ☐ No

Number

Country

Day/Month/Year filed

☐ Yes ☐ No

Number

Country

Day/Month/Year filed

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

(U.S. Serial No.) (Filing date) (Status-patented, pending, abandoned)

(U.S. Serial No.) (Filing date) (Status-patented, pending, abandoned)

(3)

And I hereby appoint as principal attorney, William M. Blackstone, Registration No. 29,772, Mary E. Gormley, Registration No. 34,409, and Michael G. Sullivan, Registration No. 35,377.

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Rockville, MD 20850-4373

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor ¹⁻⁰⁰ Andreas, Martinus, Maria MILTENBURG

Inventor's signature [Signature] ^{03 JAN 2001}
Date

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Residence and P.O. Address ^{03 JAN 2001} Hot Eiland 43 ^{KE/M 03 JAN 2001} Statenborg 19, 5346 XF OSS - The Netherlands NLX

Full name of second joint inventor ²⁻⁰⁰ Anna, Maria, Helena BOOTS

Inventor's signature [Signature] ^{03 jan 2001}
Date

Citizenship Dutch

Residence and P.O. Address Verlengde Torenstraat 10, 5366 AV MEGEN - The Netherlands

Full name of third joint inventor NLX

Inventor's signature _____
Date

Citizenship _____

Residence and P.O. Address _____

Full name of forth joint inventor _____

Inventor's signature _____
Date

Citizenship _____

Residence and P.O. Address _____